

A DNA fragment homologous to F₁-ATPase β subunit was amplified from genomic DNA of *Methanosarcina barkeri*

Indication of an archaebacterial F-type ATPase

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Received 28 September 1992; revised version received 23 October 1992

A 490 bp DNA fragment was amplified from *Methanosarcina barkeri* genomic DNA by the polymerase chain reaction (PCR) using oligonucleotide primers designed based on conserved amino acid sequences of the F₁-ATPase β subunits. The amino acid sequence deduced from the DNA sequence of this fragment was highly homologous to a portion of the F₁-ATPase β subunit. This indicates that this archaebacterium has a gene of F-type ATPase in addition to a gene of V-type ATPase.

Archaeobacteria; *Methanosarcina barkeri*; ATPase; V-type ATPase; F-type ATPase in archaeobacteria

1. INTRODUCTION

The H⁺-ATPase synthesizes ATP using an electrochemical proton gradient generated by an electron transport chain and has been considered to play an essential role in energy metabolism. Until recently it was thought that H⁺-ATPases from any organisms have the similar structural and functional features and they are designated as F₀F₁-ATPase. However, this concept was modified by the finding that ATPases from archaeobacteria [1], such as *Methanosarcina barkeri* [2,3], *Sulfolobus acidocaldarius* [4,5], and *Halobacterium halobium* [6], are only distantly related to F₀F₁-ATPase and, instead, are highly similar to eukaryotic vacuolar H⁺-ATPases [7]. It is now generally accepted that F₀F₁-ATPase and vacuolar ATPase are two subclasses of a superfamily of ATPases which do not form phosphorylated enzyme intermediates and that the former, now often called as F-type ATPase, distributes among mitochondria, chloroplasts, and eubacteria and the latter, V-type ATPase, does among archaeobacteria and eukaryotic vacuolar systems. Agreement of taxonomic border and distribution of F- and V-type ATPases has stimulated the argument for archaebacterial origin of eukaryotic vacuolar systems. However, it was soon found that distribution of V-type ATPase is not restricted to archaeobacteria: a thermophilic eubacterium *Thermus thermophilus* has a V-type

ATPase [8,9] and an eubacterium *Enterococcus hirae* has both types of ATPase [10,11]. These facts raise the possibility that restricted distribution of F-type ATPases in eubacteria and V-type ATPases in archaeobacteria is not as strict as it used to be thought and that some archaeobacteria may have V-type ATPase. In this communication, we report the amplification of a DNA fragment homologous to the F-type ATPase β subunit by the polymerase chain reaction (PCR) from *Methanosarcina barkeri* genomic DNA.

2. EXPERIMENTAL

2.1. DNA source and synthetic oligonucleotides

M. barkeri was a kind gift from Dr. Y. Koga, University of Occupational and Environmental Health. Genomic DNA from this organism was prepared as described in [12]. Oligonucleotide primers used for PCR and for DNA sequencing were synthesized using a DNA synthesizer (Applied Biosystem 381A). Three oligonucleotide primers (FL1, FL2 and FR) for PCR amplification were designed based on highly conserved regions of reported amino acid sequences of F-type ATPase β subunits. Specific features of their design are given in section 3. The primers for DNA sequencing are FL3 (5'-ATATCTAGAACGTAAGTCTGTAAGG-3') and FR3 (5'-ATTGAATTCATCAGCAGCAGGAACATA-3'). Underlined bases are the added restriction sites, *Xba*I and *Eco*RI, respectively, and sequences on the 3' side of the restriction sites are the same as one of the degenerate sequences of PCR primers, FL2 and FR, respectively.

2.2. Polymerase chain reaction

The reaction mixture (100 μ l) for PCR contained 200 ng of template (*M. barkeri* genomic DNA), 1 μ M of each primer (FR1 and FL1), 1 mM MgCl₂, 2.5 units *Taq* DNA polymerase (Promega) in the buffer provided by a Promega kit (50 mM KCl, 10 mM Tris-HCl, pH 9.0 at 25°C, 0.1% Triton X-100). PCR reactions were carried out for 1 min at 94°C, 1 min at 60°C, 1 min at 74°C and were repeated for 35 cycles

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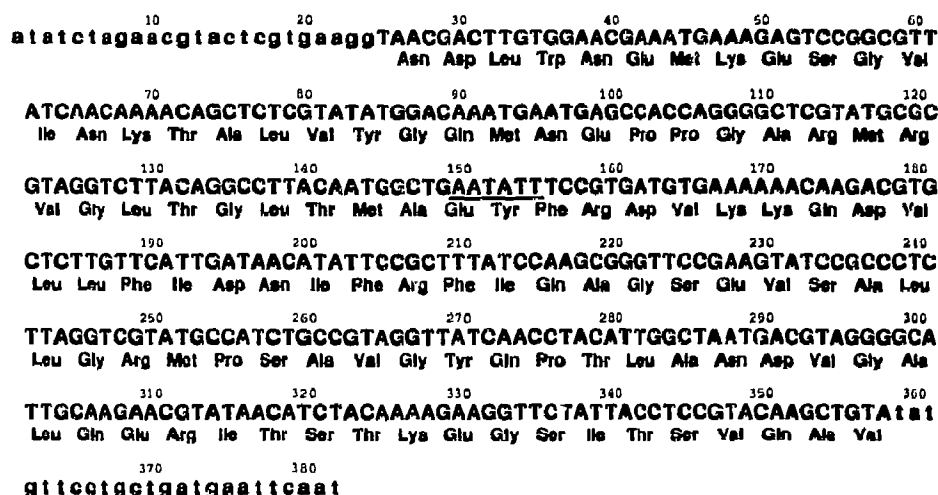


Fig. 3. Partial nucleotide sequence of the amplified 380 kbp DNA fragment and deduced amino acid sequence. The underlined sequence is the restriction site of *SspI*.

Consistently, when the amplified fragment was digested with *SspI*, it was divided into two fragments (Fig. 2, lane 4). The deduced amino acid sequence shows strong similarity to the equivalent region of F-type ATPase β subunits but not to V-type ATPases 57K subunits (Fig. 4). Therefore, we conclude that the amplified DNA fragment is a part of the F_1 -ATPase β subunit gene.

3.4. Southern blotting

Since we have repeated the whole procedures using different cultures of *M. barkeri* and obtained the fragment with the same base sequence each time, the possibility that the amplification was due to accidentally contaminating DNA is very small. Furthermore, *M. barkeri* genomic DNA digested with *EcoRI*, *HindIII* or *SspI*

was subjected to Southern blot analysis using the 380 bp DNA fragment as a probe. As shown in Fig. 5, *M. barkeri* genomic DNA digested with *EcoRI* and *HindIII* had a single positive band. Consistent with the determined base sequence of the 380 bp fragment, two positive bands appeared when the DNA was digested with *SspI*. Therefore, the possibility that the fragment was amplified from contaminated DNA is very unlikely.

4. DISCUSSION

The F-type ATPases have been found in a variety of eubacterium and eukaryotic mitochondria and chloroplasts, but, in the archaeobacterium kingdom, they have not so far been found. We have shown the amplification

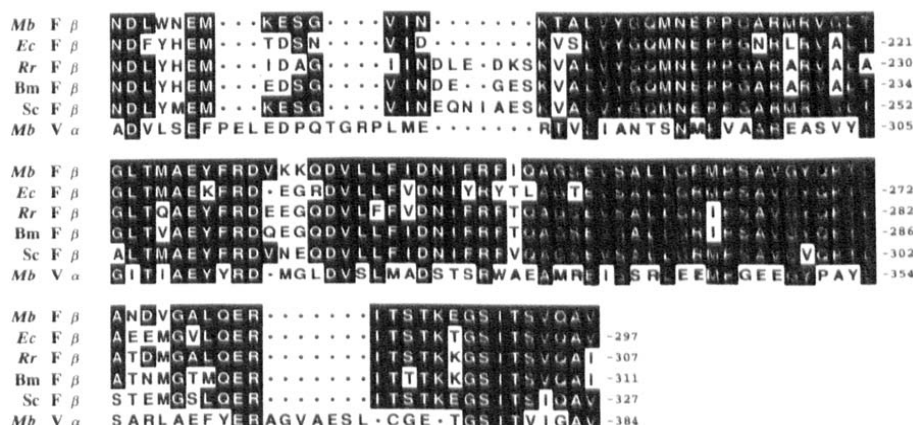


Fig. 4. Alignment of amino acid sequences of F-type ATPase β subunits from various sources and the α (57K) subunit of V-type ATPase from *M. barkeri*. The region corresponding to the amplified 380 kbp DNA fragment from *M. barkeri* (*Mb* F β) is shown. Sequence data were taken from the following references: *E. coli* (*Ec* F β , [16]), *R. rubrum* (*Rr* F β , [17]), bovine mitochondrion (*Bm* F β , [14]), spinach chloroplast (*Sc* F β , [19]). Amino acid residues identical with those from the *M. barkeri* DNA fragment are shown by white letters on a black background. The numbers at the right side of each sequence indicate those of amino acid residues. The corresponding region of *M. barkeri* V-type ATPase α subunit is also shown for reference (*Mb* V α).

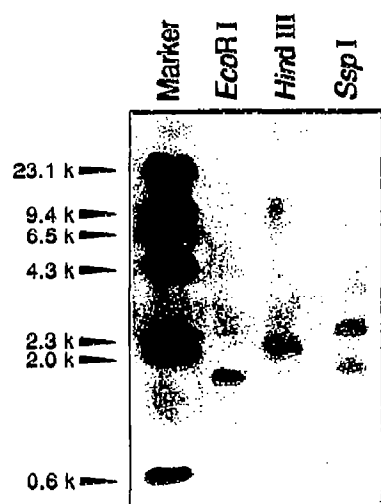


Fig. 5. Genomic southern analysis of *M. barkeri* DNA using the 380 kbp PCR fragment. *M. barkeri* genomic DNA digested with *EcoRI*, *HindIII*, or *SspI* was electrophoresed, blotted, and hybridized with the biotin-labeled amplified 380 kb DNA fragment. The biotin-labeled λ DNA digested with *HindIII* was used for the marker lane. The size of the DNA molecular weight markers (bp) are indicated.

of a DNA fragment from *M. barkeri* homologous to typical F-type ATPase β subunit genes. This suggests the strong possibility of the existence of F-type ATPase in the archaeobacterium kingdom, although cloning of a full operon or purification of the F-type ATPase from this archaeobacterium is necessary to eliminate the small possibility that it has only a part of the F-type ATPase operon and does not synthesize intact F-type ATPase. Isolation of a water-soluble part of V-type ATPase and cloning of genes encoding some of its subunits from *M. barkeri* were reported by Inatomi and his colleagues [3]. Therefore, this archaeobacterium appears to have genes of two types of ATPases. Then, two questions arise. What are the physiological roles of the two types of ATPases in the cell, and why has the presence of F-type ATPase not been recognized during procedures of V-type ATPase purification? The recent finding that a eubacterium *Enterococcus hirae* also has two types of ATPase is very interesting in this context. Kakinuma and his colleagues suggested that the F-type ATPase of *E. hirae* is a H^+ -ATPase and V-type ATPase is a Na^+ -ATPase [11]. The former is constitutively expressed but expression of the latter is induced at high Na^+ concentration in the culture medium. Probably the F-type ATPase of *M. barkeri* pumps a different ion than the

V-type ATPase and is synthesized only under the limited condition. Concerning the evolutionary aspect of this finding, it is our view that, since distribution of F-type and V-type ATPases among prokaryote kingdoms turns out to be not as simple as we used to think, exact distribution, sequence comparison, and in vivo function of F- and V-type ATPases in eubacteria and archaeobacteria should be clarified before profound argument.

Acknowledgements: We thank Dr. Y. Koga for kindly supplying *M. barkeri* cells to us.

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